BENEFICIAL INFLUENCE OF MATCHING FOR D/DR ANTIGENS AND TREATMENT WITH CYCLOSPORIN-A ON KIDNEY GRAFT SURVIVAL IN TRANSFUSED RHESUS MONKEYS

J C C Borleffs, R L Marquet*, P Neuhaus†

Primate Center TNO, Rijswijk, The Netherlands, *Erasmus University, Rotterdam, The Netherlands, and †Klinik für Abdominal und Transplantationschirurgie, Medizinische Hochschule, Hannover, FRG

Summary

The influence of matching for D/DR antigens and treatment with Cyclosporin-A (Cy-A) was investigated in rhesus monkeys given pretransplant blood transfusions. It was observed that the combination of D/DR matching and transfusions led to a higher percentage of animals with prolonged graft survival as well as a better kidney function in the early post-transplant period than was achieved with transfusions alone. Regarding the possible interference of Cy-A with the beneficial blood transfusion effect, it was found that transfused animals given Cy-A had survival times which were as good as those of transfused recipients without Cy-A or, sometimes, even slightly better.

Introduction

Clinical [1] and experimental data for rhesus monkeys [2] indicate that pretransplant blood transfusions have a clearly positive effect on kidney graft prognosis. In addition, there are other procedures which also have a beneficial influence, such as matching for D/DR antigens and treatment with Cyclosporin-A (Cy-A). In the monkey model, the favourable influence of DR matching was seen only in combinations in which there was MLC nonreactivity between cells from recipient and donor; this requires identity for the two DR antigens. Strong immunosuppressive properties of Cy-A have been described [3]. However, as it was not clear whether these two procedures would change the favourable blood transfusion effect, a controlled preclinical animal study was performed to investigate any possible interference. As far as the data for Cy-A treatment are concerned, the results presented in this communication are preliminary.

Materials and methods

Animals Young unrelated male and female rhesus monkeys (Macaca mulatta) weighing 3–5kg were used. They were imported from India or born at the
TABLE I. Influence of matching for D/DR antigens and treatment with cyclosporin-A on kidney graft survival in unrelated, transfused rhesus monkeys

<table>
<thead>
<tr>
<th>Group</th>
<th>Shared DR antigens</th>
<th>MLC reactivity R → D_X*</th>
<th>Transfusions</th>
<th>Immunosuppression †</th>
<th>Individual survival times (days)</th>
<th>Recipients with prolonged survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 – 1</td>
<td>Positive</td>
<td>–</td>
<td>Standard i.s.</td>
<td>9  9  10  10  11  12  14  14  17  22</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>0 – 1</td>
<td>Positive</td>
<td>+</td>
<td>Standard i.s.</td>
<td>9  11  12  13  13  19  22  41  43  43  53  61</td>
<td>42% (5/12)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Negative</td>
<td>+</td>
<td>Standard i.s.</td>
<td>19  27  27  32  34  39  41  43  54  73</td>
<td>90% (9/10)</td>
</tr>
<tr>
<td>4</td>
<td>0 – 1</td>
<td>Positive</td>
<td>+</td>
<td>Cy-A only</td>
<td>14  31  32  36†  36  47†</td>
<td>83% (5/6)</td>
</tr>
<tr>
<td>5</td>
<td>0 – 1</td>
<td>Positive</td>
<td>+</td>
<td>Standard i.s. + Cy-A</td>
<td>33  42†  46†  46‡  48  81‡</td>
<td>100% (6/6)</td>
</tr>
</tbody>
</table>

* R → D_X: cells of recipient versus irradiated cells of donor

† Standard i.s. consisted of azathioprine (2mg.kg⁻¹) and prednisolone (1mg.kg⁻¹).

‡ Dosage levels of cyclosporin-A were 10–25mg.kg⁻¹. Animals of groups 1–3 received i.s. therapy for a period of 45 days, those of groups 4–5 for only three weeks after transplantation.
Rijswijk Primate Center. Kinship among the imported animals cannot be excluded but is unlikely.

Kidney transplantation  Kidney grafting was performed as described previously [4]. Death attributable to graft failure was considered as the end-point of graft survival. After transplantation, blood urea, potassium and creatinine levels were regularly determined.

Transfusions  Three transfusions each consisting of 20ml of fresh, unpooled, whole citrated blood were given at biweekly intervals before transplantation, the last one two to three weeks prior to operation. The blood donors were selected in such a way that immunisation against SD (A and B locus antigens of the rhesus monkey major histocompatibility complex, RhLA) antigens for which the planned kidney donor was disparate with the recipient would be avoided as much as possible; this was done to minimise the chance of positive crossmatches at the time of transplantation.

Immunosuppression  Recipients received azathioprine, prednisolone or cyclosporin-A. Doses and duration of treatment were according to the protocol of experiments described in Table I. Drugs were administered i.m. daily starting on the day of transplantation.

Tissue typing and matching  Animals were typed for the conventional SD and DR antigens [5, 6]. In all host/donor combinations, lymphocytes of the recipients were tested in MLC against lymphocytes of the planned kidney donor and against those of unrelated control monkeys. MLC results were analysed as described earlier [7]. SD antigens were disregarded in selecting host/donor combinations.

Results and discussion

Blood transfusions and D/DR matching

The experimental groups are shown in Table I. It was found that nontransfused D/DR mismatched recipients (group 1) had survival times ranging from 9 to 22 days. Animals of the other groups surviving > 22 days were considered to show 'prolonged' graft survival. Transfused recipients had a prolonged survival time in 5 of 12 cases (42%) if not matched with their donors for D/DR antigens (group 2) and in 9 of 10 (90%) if the animals had been matched (group 3). Figure 1 shows mean serum creatinine levels of recipients in experimental groups 2 and 3. Only creatinine values of animals that survived for more than 22 days (i.e. prolonged) are included in this figure, since animals dying before day 22 showed creatinine levels of more than 400μmol.L⁻¹ as early as days 5 or 10 (normal values for rhesus monkeys are 60–100μmol.L⁻¹). The mean serum creatinine values ± SE (μmol.L⁻¹) for the two groups at days 5, 10 and 15 were as follows: for group 2 (transfused, D/DR mismatched), 146 ± 36.0, 259 ± 105.3 and 337 ±153.0; for group 3 (transfused, D/DR matched), 93 ± 9.3, 85 ± 6.3 and 96 ± 8.8. The values
for group 3 were significantly lower than those for group 2 (p < 0.05 by Mann-Whitney U test).

These data show that there is an additive beneficial effect of matching for D/DR antigens on kidney graft survival in transfused rhesus monkeys. Matching for D/DR clearly led to a higher percentage of recipients with prolonged graft survival as well as better kidney function in the early post-transplant period. It seems reasonable to assume that, in transfused animals, the number of rejection episodes will be smaller in D/DR matched host/donor combinations than in D/DR mismatched pairs. In man, the administration of pretransplant blood transfusions and matching for DR antigens are currently accepted procedures; both show a positive influence on kidney graft prognosis. Somewhat surprisingly, as is evident from several clinical studies, combination of these procedures has not resulted in
an additive effect [1, 8]. This seems in disagreement with the additive effect found in the current monkey study. In the monkey experiments, however, matching for DR as well as for D antigens was done; therefore, MLC nonresponsiveness could be analysed for an effect which was possibly additive to that of transfusions. In the clinical situation, on the other hand, prospective matching for D antigens (and thus for MLC nonresponsiveness) is usually not possible because of the time required for the test (four to five days). One clinical team was able to analyse their DR matched combinations for D compatibility. They found a beneficial effect of matching for D in transfused recipients [9]. These findings and our monkey data are in accord. Further monkey experiments (now in progress) suggest that recipients given transfusions followed by kidneys from donors matched for DR but not for D (MLC responsive) have a less favourable prognosis than when matching for both D and DR is done. Thus, pretransplant blood transfusions combined with matching for D and DR antigens seems to be an excellent protocol for the monkey and presumably also for man.

**Blood transfusions and cyclosporin-A**

The experimental groups are shown in Table I. Transfused recipients which received Cy-A only (group 4) had a prolonged survival time in five of six cases (83%); this was 100% if the animals had been additionally treated with standard immunosuppression (i.s.) (group 5). Recipients given the combined therapy survived for significantly longer times than animals which received Cy-A only (p < 0.02 by Mann-Whitney U test). It is important to note that monkeys of these two experimental groups were treated with the immunosuppressive drugs for only three weeks. There were no signs of toxicity or infection and no abnormalities or tumours were observed at autopsy, even when Cy-A was combined with standard i.s.; this might be due to the fact that the period of treatment was too short for observing side-effects.

Although these data are preliminary, they suggest that, in transfused rhesus monkeys, Cy-A is more effective than azathioprine and prednisolone (group 2). The percentage of recipients with prolonged survival times is far higher in Cy-A treated animals than in monkeys given standard i.s. Therefore, it was found that Cy-A did not diminish the positive blood transfusion effect but sometimes even slightly improved the effect.

**Conclusions**

The administration of pretransplant blood transfusions is one of the most important procedures for obtaining long surviving kidney allografts. However, a beneficial effect cannot be found in all experimental animals. This study indicates that host/donor matching for D/DR antigens increases the rate of success of transplantation in transfused recipients. Yet, preliminary data on Cy-A show that, with only a short-term period of treatment with this drug, impressive results can be obtained. It can be speculated that in the future the combination of pretransplant blood transfusions and the administration of Cy-A, will be sufficient for obtaining long surviving kidney allografts.
Acknowledgments

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Open Discussion

BARNES (Birmingham) Those are very nice studies but what you have not shown is any data related to D/DR matching without transfusion. Have you got any data on that? You claim they are additive but you have only given your D/DR matched results in transfused patients, not in non-transfused patients.

BORLEFFS They are not shown in this presentation, I agree with you, but we performed these transplantations too. There was a prolonged survival time in 62% of the animals if they were not transfused and only matched with D/DR antigens and the same significant creatinine differences were found. So in fact they behaved like mismatched-transfused combinations.

BARNES So the two things are equivalent.

BORLEFFS Matching for D/DR or transfusions is in this animal equivalent and if you combine these two procedures the effect is additive.

BARNES The other question was that if you give your major immunosuppression with azathioprine, prednisolone and cyclosporin in the non-transfused what happens then?

BORLEFFS These experiments are in progress and we have done just a few of them, but the impression is gained that when giving cyclosporin A, whether or not in combination with standard immunosuppression, to non-transfused D/DR mismatched animals, the results are comparable with the long surviving
transfused D/DR mismatched or the long surviving non-transfused D/DR matched. All of them are up to now showing a prolonged survival time.

BARNES I was just rather surprised that you selected out only those few samples to show us and did not give the whole data.

BORLEFFS That is because the data is preliminary.

BARNES So it is not a randomised series; there is a fair amount of selection going on.

BORLEFFS This is part of a big programme we are involved with, the one part of the programme is cyclosporin-A treatment in non-transfused animals and another part is continuous treatment with cyclosporin-A in transfused animals. Unfortunately I cannot present all this data to you because the study is in progress.

BACH (Co-Chairman) Do you have any information about the immunological events which take place after transfusion, in other words, what is the possible immunological basis of the transfusion effect? Do you have any data suggesting that the transfusion effect could take place at the level of DR antigen rather than for the monkey equivalent of the A and B antigens?

BORLEFFS That is really difficult to answer. We got the impression, but it does not explain the blood transfusion mechanism, that in MLC positive combinations which are positive before the first transfusion, if the recipients receive three pre-transplantation blood transfusions some of the monkeys will have a lower mixed lymphocyte culture against the transplant kidney donor. Some of them will be higher, some unchanged, but the monkeys with an increased MLC response are short survivors. But that does not explain the mechanism, I agree.

BRUNNER (Basle) Could you help me with the meaning of DR matching in your monkeys. I think DR matching in human cadaver transplantation is completely different from the MLC matching in your rhesus monkeys, is it not?

BORLEFFS Yes it is. Matching for DR in man is the same as matching for DR in the monkeys but we could analyse our combinations in an extra respect, matching for D just because it is a prospective animal study. I agree that these monkeys were not only matched for DR but also for the D antigens.

BRUNNER Do you find monkeys matched for D that are not matched for DR?

BORLEFFS No. We do find monkeys which are matched for DR but not for
D, but we do not find monkeys which are matched for D (that means MLC non-responsive) but not matched for DR.

BRUNNER I think you are giving us a wrong impression. What you did was matching for D.

BORLEFFS I think also for DR.

BRUNNER You just did it, but that does not mean anything.

BORLEFFS Matching for DR makes it much more easy to find MLC unresponsive combinations.

BRUNNER Yes, but I think you cannot transfer this information to human cadaver kidney transplantation.

VAN YPERSELE (Chairman) Unfortunately we shall have to conclude on the fact that your D and DR are so related that you cannot distinguish between both, and Felix Brunner says that it is really not the case in human beings.